

Synthesis and Anti-inflammatory Activity of New *N*-Acyl-2-pyrazolines Bearing Homologous Alkyloxy Side Chains

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Abstract

A series of new pyrazoline derivatives (**1a-2h**) equipped with *N*-acyl arms and homologous alkyloxy side chains were synthesized and characterized on the basis of spectroscopic data and microanalysis. All the synthesized compounds were screened for their *in-vitro* anti-inflammatory activity to examine the effect of alkyloxy side chain length on activity. Compounds with odd number of carbons in alkyloxy side chain showed better activity as compared to even ones. Compound **2c** (96% inhibition, $IC_{50} = 173.06 \pm 2.312$ mM) was found to be the most active compound of the series with better activity than the standard (Indomethacine, 92% inhibition, $IC_{50} = 273.12 \pm 2.33$ mM). Compound **1a** (86%, $IC_{50} = 296.16 \pm 2.091$ mM) was the second best with comparable activity to the standard drug. However, the other compounds of series showed moderate to low activity. Interestingly, parallel cytotoxicity studies of compounds **1a-2h** against PC-3 cell line revealed either no or very low cytotoxicity. The study may contribute in developing useful alternatives to presently used NSAIDs with harmful gastric effects due to direct cytotoxicity.

Keywords: *N*-Acyl-2-pyrazolines, synthesis, anti-inflammatory, cytotoxicity

1. Introduction

Pyrazolines being a member of five membered heterocycles family, represent a class of compounds having immense importance in heterocyclic chemistry. Pyrazoline is a dihydropyrazole having two nitrogen atoms in adjacent positions and possessing only one endocyclic double bond. Considerable interest on the pyrazoline structure has been focused in the field of medicinal chemistry. Among all the pyrazolines, 2-pyrazoline has gained much attention due to its broad spectrum biological activities¹⁻³ such as antiamebic,⁴⁻⁷ antimycobacterial,⁵ antibacterial/antifungal,⁶ anti-inflammatory,⁸ anticancer,^{9,10} antidepressant,^{11,12} neuroprotector,^{13,14} antiviral^{15,16} and antiobesity¹⁷ and its presence in a number of pharmacologically active molecules such as azolid/ tandearil (anti-inflammatory), phenazone/ amidopyrene/ methampyrone (analgesic and antipyretic), anturane (uricosuric) and indoxacarb (insecticidal). Although, a number of pyrazoline-based new compounds have been made and patented in recent years possessing diverse biological activities¹⁻³ but

still it is an active area of research¹⁸⁻²³ and many new aspects need to be explored and worked on.

Inflammation occurs as a defensive biological response of vascular tissues to harmful stimuli resulting in some physiological adaptations to minimize tissue damage and initiate the healing process.²⁴⁻²⁶ Non-steroidal anti-inflammatory drugs (NSAIDs) are usually used to treat inflammation. The delay in treatment may lead to vasomotor rhinorrhoea, rheumatoid arthritis, and atherosclerosis.²⁷ NSAIDs inhibit the activity of both cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) which are the key enzymes involved in the biosynthesis of prostaglandin from arachidonic acid.^{28,29} Such inhibition reduces the levels of protection resulting in harmful gastric effects.³⁰ Therefore, most of the presently used NSAIDs are not very useful in all inflammatory disorders.³¹ However, it has recently been found that harmful gastric effects of NSAIDs are not related to PGE2 inhibition but are rather due to the direct cytotoxicity in the stomach to gastric cells.^{32,33} Therefore, new anti-inflammatory agents with no/less such adverse gastric effects are needed. As a conti-

uation of our ongoing project on synthesis and applications of pyrazolines,^{34–37} herein, we report the synthesis of some new anti-inflammatory *N*-acyl pyrazolines bearing homologous alkoxy side chains with almost negligible cytotoxicity.

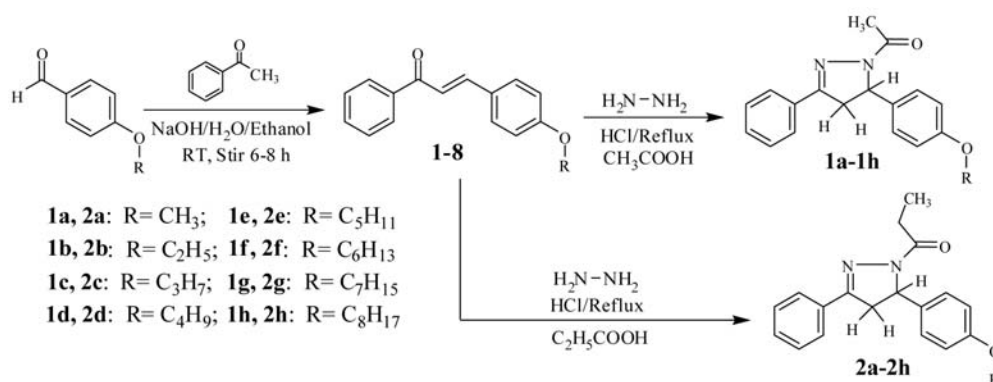
2. Results and Discussion

2.1. Chemistry

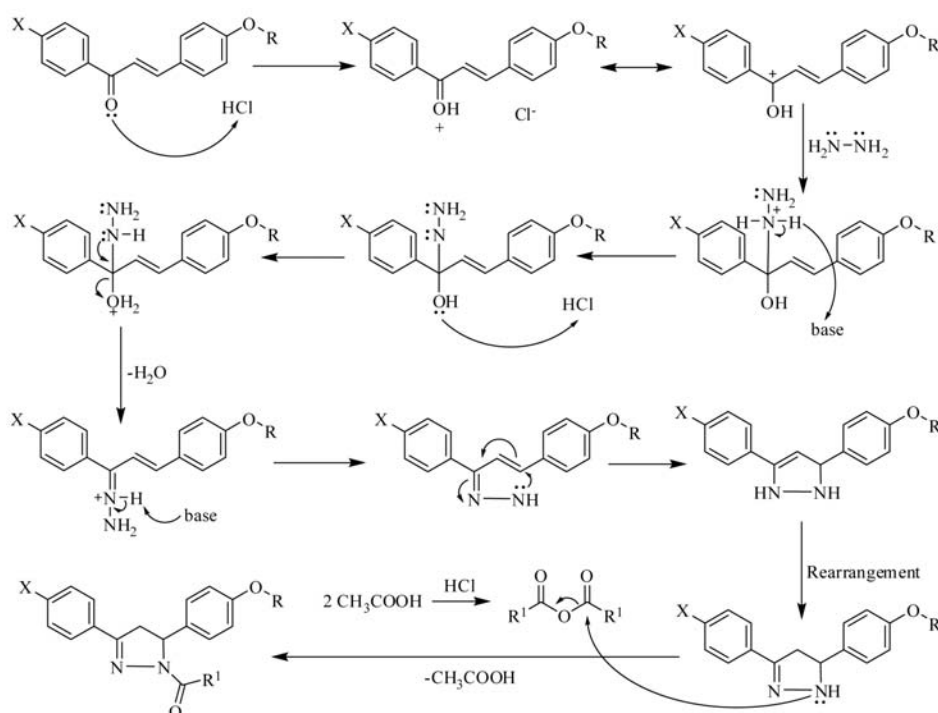
The compounds **1a–2h** were synthesized by refluxing an equimolar mixture of (*E*)-3-(4-alkoxyphenyl)-1-phenylprop-2-en-1-ones (**1–8**)³⁸ and hydrazine in the respective solvents such as glacial acetic acid and propionic acid containing catalytic amount of hydrochloric acid (Scheme 1 & 2) and purified by silica gel column chroma-

tography using petroleum ether/ethyl acetate (4:1) as mobile phase. All the products were obtained as solids in 81–89% yield. The structures of all the synthesized compounds were deduced by their spectroscopic (IR, ¹H NMR & ¹³C NMR) and elemental analyses data.

In the IR spectra of compounds (**1a–2h**), a sharp band at 1647–1633 cm⁻¹ and 1688–1675 cm⁻¹ was assigned to the stretching of $\nu(\text{C}=\text{N})$ and $\nu(\text{C}=\text{O})$, respectively.^{34–37,39–41} The carbon-nitrogen single bond (C–N) stretching frequencies were observed at 1298–1290 cm⁻¹. The presence of these frequencies suggests the formation of cyclization product. Two strong bands at stretching frequencies in the range of 1259–1250 cm⁻¹ and 1056–1042 cm⁻¹ indicate the presence of Ar–O–R group. The formation of the five membered pyrazoline ring was further confirmed by the presence of three doublet of doublets due to



Scheme 1. Synthesis of 2-functionalised pyrazolines **1a–2h**.



Scheme 2. Proposed mechanism for the synthesis of 2-functionalised pyrazolines **1a–2h**

two methylene protons (H_a and H_b) and one methine proton (H_x) in 1H NMR spectroscopy. The methyl protons of acetyl group ($O=C-CH_3$) were observed at 2.43 ppm as singlet for compounds **1a–1h**, whereas a triplet and quartet at 1.20–1.22 and 2.83–2.84 ppm was noticed for methyl protons ($O=C-CH_2-CH_3$) and the methylene protons ($O=C-CH_2-CH_3$), respectively for propionyl group of compounds **2a–2h**. A triplet was observed in the range of 3.79–4.00 ppm for the methylenic protons ($Ar-O-CH_2-$) of the alkoxy chain directly attached to the oxygen atom. All the other protons of alkoxy chain appeared in the range of 0.90–1.77 ppm. The ^{13}C NMR spectra of compounds (**1a–2h**) displayed peaks at 55–65 ppm, 59–70 ppm and 41–44 ppm for C_5 , C_3 and C_4 carbons, respectively. All the aromatic carbons were observed in the range of 114–159 ppm. The signals for acyl carbons were noticed in the range of 167–173 ppm. Furthermore, a molecular ion peak (M^+) for each compound was also observed at their respective masses along with their typical pyrazoline fragmentation pattern.⁴² In addition, the number of the protons and carbons were found in good agreement with elemental (CHN) analyses suggesting the formation of the target compounds. The structures of the two compounds **1a**⁴³ and **1f**⁴⁴ were unambiguously confirmed through single crystal X-Ray diffraction technique (Figure 1).

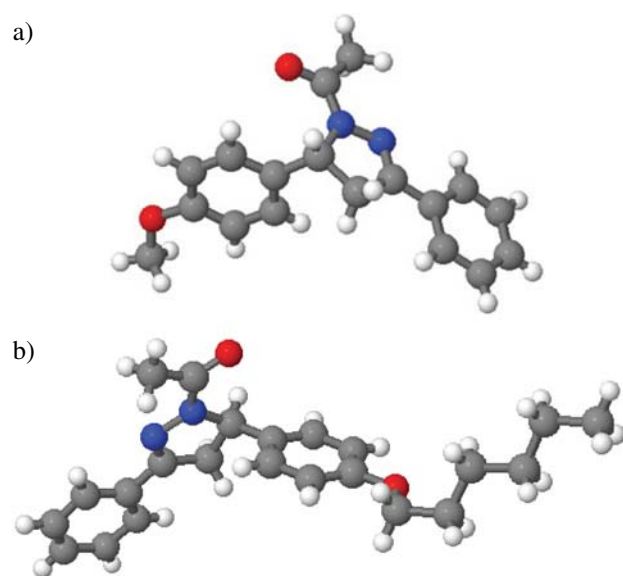


Figure 1. (a) Crystal Structure of **1a**.⁴³ (b) Crystal Structure of **1f**.⁴⁴

2. 2. Antiinflammatory Activity (in vitro)

The defensive process of host in response to foreign challenge or tissue injury for restoration of normal tissue structure and function is initiated by the activation of phagocyte-specific enzyme, NADPH oxidase which generates superoxide anion (a reactive free radical) by transferring electrons from NADPH to molecular oxygen insi-

de the cell across the membrane. The generated superoxide then kills bacteria and fungi by still unknown mechanisms. This is the key step of immune response and inflammatory cascade. However, this superoxide may lead to the formation of hydrogen peroxide which is capable of undergoing further reactions to produce highly toxic reactive oxygen species (ROS). In immune compromised patients, ROS are formed in large quantities. Therefore, their inhibition is one approach to treat chronic inflammation and to modulate immune response.

This study used the water-soluble tetrazolium salt (WST-1) to measure superoxide production by neutrophils activated by opsonized zymosan, which induces phagocytic activation of neutrophils. This technique is more sensitive and reliable to measure the superoxide scavenging properties as compared to other available techniques, a perfect protocol for indirect evaluation of anti-inflammatory potential.⁴⁵ Using this technique, the anti-inflammatory potential of the *N*-acyl-2-pyrazolines **1a–2h** bearing homologous alkoxy side chains was determined in terms of percent inhibition. The compounds with ~50% inhibition were retested for their IC_{50} (inhibitory concentration 50%), the concentration of the compound which inhibits superoxide production by 50% of three independent experiments (Table 1, Figure 2). Indomethacine was used as the standard reference drug in this study. The compounds (**1a–2h**) showed a varying degree of anti-inflammatory activity, when compared to standard drug. Compound **2c** (96%, 173.06 ± 2.312) showed excellent anti-inflammatory activity even better than the standard (92%, 273.12 ± 2.33). Compound **1a** (86%, 296.16 ± 2.091) also exhibited good and comparable anti-inflammatory activity. Compound **1e** (55%, 465.23 ± 1.763), **1h** (52%, 492.20 ± 3.176), **2e** (76%, 346.13 ± 2.341) and **7c** (51%, 425.21 ± 2.732) showed moderate anti-inflammatory potential. However, compound **1b**, **1c**, **1d**, **1f**, **1g**, **2a**, **2b**, **2d**, **2f** and **2h** were considered to be least active compound among the series having less than 50% inhibition and were not further tested for their IC_{50} values.

This varying degree of anti-inflammatory activity of 16 tested compounds (**1a–2h**) can be attributed to alkoxy side chain length because central nucleus *N*-acyl-2-pyrazolines is the same for all the compounds. Surprisingly, compounds with odd number of carbons in alkoxy group showed maximum activity as compared to the compounds having even numbered alkoxy side chain (Table 1, Figure 2). For example, compound **1a** (86%, 296.16 ± 2.091) and compound **2c** (96%, 173.06 ± 2.312) with methoxy and propyloxy substituents, respectively showed maximum activity. This difference in activity with the change in alkoxy side chain length in compound (**1a–2h**) may be credited to some specific conformational arrangements of alkoxy side chains due to different amount of non-covalent interactions in their packed state. The precise mechanism of inhibitory action of these compounds is now under investigation.

Table 1. Anti-inflammatory Activity of 1a–2h.

Compd.	R ¹	R ²	% Inhibition and IC ₅₀		Profile Activity
			% Inhibition (at 500 μ M)	IC ₅₀ (μ M)	
1a	CH ₃	CH ₃	86	296.16 $\pm$ 2.091	Good
1b	C ₂ H ₅	CH ₃	32	–	Weak
1c	C ₃ H ₇	CH ₃	21	–	Weak
1d	C ₄ H ₉	CH ₃	43	–	Weak
1e	C ₅ H ₁₁	CH ₃	55	465.23 $\pm$ 1.763	Moderate
1f	C ₆ H ₁₃	CH ₃	11	–	Weak
1g	C ₇ H ₁₅	CH ₃	17	–	Weak
1h	C ₈ H ₁₇	CH ₃	52	492.20 $\pm$ 3.176	Moderate
2a	CH ₃	C ₂ H ₅	15	–	Weak
2b	C ₂ H ₅	C ₂ H ₅	06	–	Weak
2c	C ₃ H ₇	C ₂ H ₅	96	173.06 $\pm$ 2.312	Excellent
2d	C ₄ H ₉	C ₂ H ₅	42	–	Weak
2e	C ₅ H ₁₁	C ₂ H ₅	76	346.13 $\pm$ 2.341	Moderate
2f	C ₆ H ₁₃	C ₂ H ₅	32	–	Weak
2g	C ₇ H ₁₅	C ₂ H ₅	51	425.21 $\pm$ 2.732	Moderate
2h	C ₈ H ₁₇	C ₂ H ₅	22	–	Weak
INDOM ^[a]	–	–	92	273.12 $\pm$ 2.33	Standard

^[a] INDOM : Indomethacine (Standard Drug).

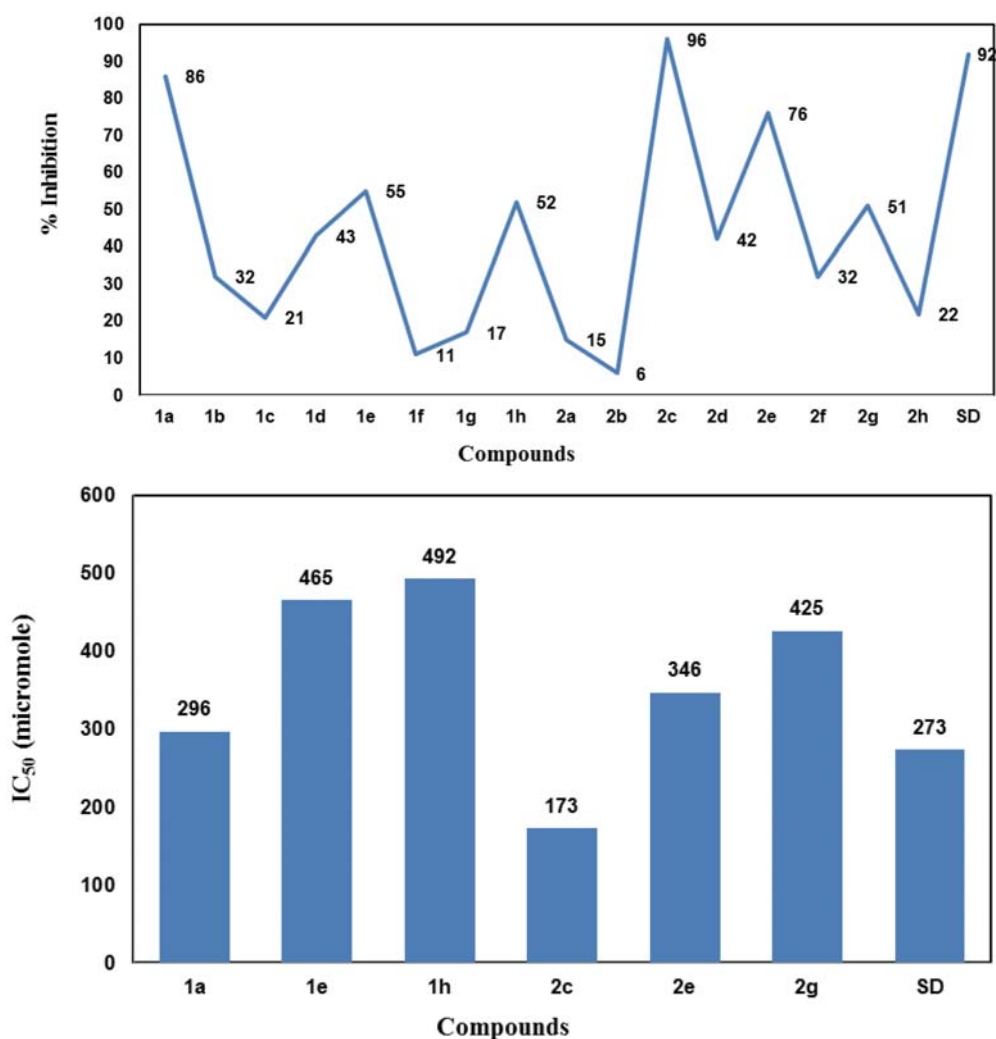


Figure 2: Anti-inflammatory activity of 1a–2h, % inhibition (top) and IC₅₀ (bottom) of compounds with more than 50% inhibition. SD = Indomethacine

2. 3. Cytotoxicity Assay Against Prostate Cancer (PC-3 Cell Line)

The newly synthesized compounds **1a–2h** were initially screened at the single concentration of 100 μM using the colorimetric MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] to test their *in vitro* cytotoxicity,⁴⁶ against human prostate cancer cell line (PC3). Doxorubicin was used as the standard reference drug in this study. The cytotoxicity of tested compounds was estimated in terms of percent growth inhibition compared to untreated control cells. Then, the compounds effecting ~70% inhibition in one dose prescreening were retested by serial

dilution from 100–20 μM . The results were expressed as IC_{50} (inhibitory concentration 50%), the concentration of the compound which inhibits the tumor cell growth by 50% and the data are presented in Table 2 & Figure 3.

Close inspection of the acquired cytotoxic data revealed that almost all of the tested compounds showed no/very low cytotoxicity against the PC-3 tested cell line. The compounds **1b**, **1h**, **2a**, **2b**, **2c** and **2e** were found to be slightly toxic against the PC-3 tested cell line with IC_{50} values ranging from 27–67 μM compared to DOX, IC_{50} (0.912 μM) whereas the other compounds showed almost no cytotoxicity. The results of this study indicate that compounds **1a** and **2c** of this series having anti-inflamma-

Table 2. Cytotoxicity bioassay of compounds **1a–2h** against prostate cancer cell line (PC-3).

Compd.	R ¹	R ²	IC ₅₀ (μM)	Compd.	R ¹	R ²	IC ₅₀ (μM)
1a	CH ₃	CH ₃	>100	2a	CH ₃	C ₂ H ₅	41
1b	C ₂ H ₅	CH ₃	57	2b	C ₂ H ₅	C ₂ H ₅	29
1c	C ₃ H ₇	CH ₃	>100	2c	C ₃ H ₇	C ₂ H ₅	27
1d	C ₄ H ₉	CH ₃	>100	2d	C ₄ H ₉	C ₂ H ₅	>100
1e	C ₅ H ₁₁	CH ₃	>100	2e	C ₅ H ₁₁	C ₂ H ₅	67
1f	C ₆ H ₁₃	CH ₃	>100	2f	C ₆ H ₁₃	C ₂ H ₅	>100
1g	C ₇ H ₁₅	CH ₃	>100	2g	C ₇ H ₁₅	C ₂ H ₅	>100
1h	C ₈ H ₁₇	CH ₃	42	2h	C ₈ H ₁₇	C ₂ H ₅	>100
DOX ^[a]	—	—	0.912	DOX	—	—	0.912

^[a] DOX: Doxorubicin (Standard Drug)

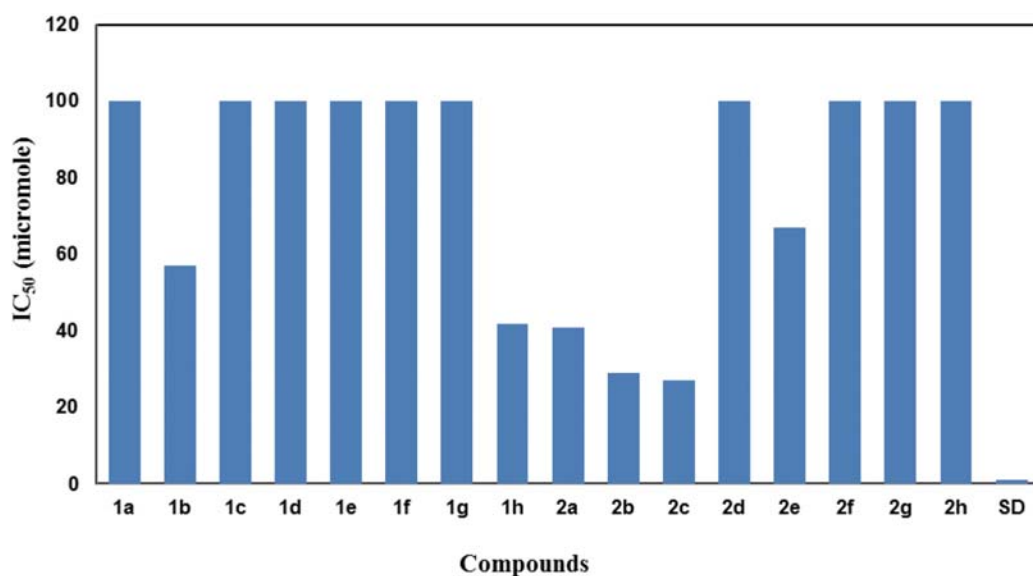


Figure 3. Cytotoxicity of **1a–2h** against PC-3 cell line, SD = Doxorubicin

tory activity better than and comparable to standard drug, and with no/little cytotoxicity are promising future candidates for further anti-inflammatory research to minimize harmful gastric effects, usually originate from direct cytotoxicity of NSAIDs.

3. Conclusions

In conclusion, we have presented the synthesis and anti-inflammatory activity of some new *N*-acyl arms & homologous alkyloxy side chains bearing pyrazoline derivatives (**1a–2h**). In general, compounds with odd carbon alkyloxy side chain showed better activity. Compound **2c** (96% inhibition, $IC_{50} = 173.06 \pm 2.312 \mu\text{M}$) and **1a** (86%, $IC_{50} = 296.16 \pm 2.091 \mu\text{M}$) showed maximum activity with **2c** even better than the standard drug, Indomethacin, (92% inhibition, $IC_{50} = 273.12 \pm 2.33 \mu\text{M}$). Parallel cytotoxicity studies on Prostate cancer (PC-3) cell line demonstrated no or very low activity for all the compounds. It has been shown recently that harmful gastric effects of NSAIDs are not related to PGE2 inhibition but are rather due to the direct cytotoxicity in the stomach to gastric cells.^{32,33} The compounds of the present series may therefore be promising replacement anti-inflammatory agents to existing NSAIDs having harmful gastric effects due to direct cytotoxicity and merits further research. This study also insinuates constructive hints for the design of new effective anti-inflammatory agents having either no or very low cytotoxicity to gastric cells.

4. Experimental Protocols

4.1. Materials and Methods

All reagents and solvents were used as obtained from the supplier or recrystallized/redistilled as required. Thin layer chromatography was performed using aluminium sheets (Merck) coated with silica gel 60 F₂₅₄. Elemental analyses were carried out with a LECO-183 model. ¹H and ¹³C NMR spectra of compounds were recorded with a Bruker 300 MHz spectrometer using deuterated solvents and TMS as internal standard. IR spectra of compounds were recorded on a Bio-Rad FTS 3000 MX spectrophotometer

(400–4000 cm⁻¹). The melting points of compounds were determined using capillary tubes and an electrothermal melting point apparatus, model MP-D Mitamura Riken Kogyo, Japan. In vitro anti-inflammatory, antifungal and cytotoxic properties were studied at HEJ research Institute of Chemistry, International Center for Chemical Sciences, university of Karachi, Pakistan.

4.2. General procedure for the synthesis of compounds (**1a–2h**)

The carboxylic acid (25 mL) solution of the respective 4-alkoxychalcone (**1–8**)³⁸ (0.01 mole), hydrochloric acid (5–7 drops) was heated at 60–65 °C for 30 minutes with constant stirring. Hydrazine hydrate (80%) (1.0 g, 0.02 mole) was then added dropwise to the reaction flask and the reaction mixture was refluxed for about 4–5 hour before cooling it to room temperature and adding crushed ice into it to get the precipitates. The precipitates so obtained were filtered, washed with distilled water and dried, and further purified by column chromatography using silica gel and petroleum ether/ethyl acetate (4:1) as mobile phase to get pure **1a–2h** in 81–89% yields (Figure 4).

1-Acetyl-3-phenyl-5-(4-methoxyphenyl)-2-pyrazoline (**1a**)

Yellowish white crystals. Yield 85%. M. p. 123–125 °C; $R_f = 0.68$ (petroleum ether:ethyl acetate, 4:1), FT-IR (KBr, cm⁻¹): 1682, 1637, 1495, 1298, 1255, 1046, ¹H NMR (300 MHz, CDCl₃) δ 2.43 (s, 3H, O=C-CH₃), 3.18 (dd, 1H, $J = 4.5, 17.7$ Hz, H_a), 3.75 (dd, 1H, $J = 11.7, 17.7$ Hz, H_b), 3.79 (s, 3H, -O-CH₃), 5.57 (dd, 1H, $J = 4.5, 11.7$ Hz, H_c), 6.86 (d, 2H, $J = 8.7$ Hz, ArH_{c-e}), 7.19 (d, 2H, $J = 8.7$ Hz, ArH_{d=f}), 7.44–7.47 (m, 3H, ArH_{f=g}), 7.74–7.79 (m, 2H, ArH_{e=h}), ¹³C NMR (75 MHz, CDCl₃) δ 22.0, 42.2, 55.2, 59.4, 114.2 (2C), 126.5 (2C), 126.9 (2C), 128.7 (2C), 130.3, 131.4, 134.1, 153.8, 159.0, 168.8, (EI) m/z (M^{++} 294, Base Peak 251); Anal. calcd. For C₁₈H₁₈N₂O₂: C, 73.45; H, 6.16; N, 9.52; Found: C, 73.49; H, 6.14; N, 9.59 %.

1-Acetyl-3-phenyl-5-(4-ethoxyphenyl)-2-pyrazoline (**1b**)

Yellowish white crystals. Yield 89%. M. p. 116–118 °C; $R_f = 0.70$ (petroleum ether:ethyl acetate, 4:1), FT-IR (KB-

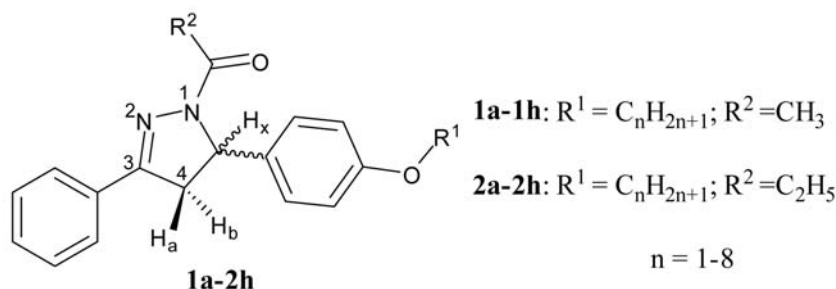


Figure 4. Labelling scheme of protons of compounds **1a–1h**.

r, cm^{-1}) 1678, 1632, 1499, 1295, 1257, 1042, ^1H NMR (300 MHz, CDCl_3) δ 1.40 (t, 3H, $J = 7.0$ Hz, $-\text{O}-\text{CH}_2-\text{CH}_3$), 2.43 (s, 3H, $\text{O}=\text{C}-\text{CH}_3$), 3.18 (dd, 1H, $J = 4.5, 17.7$ Hz, H_a), 3.75 (dd, 1H, $J = 11.7, 17.7$ Hz, H_b), 4.0 (q, 3H, $J = 6.9$ Hz, $-\text{O}-\text{CH}_2-$), 5.57 (dd, 1H, $J = 4.5, 12.0$ Hz, H_x), 6.85 (d, 2H, $J = 8.7$ Hz, $\text{ArH}_{c=c'}$), 7.17 (d, 2H, $J = 8.7$ Hz, $\text{ArH}_{d=d'}$), 7.43–7.47 (m, 3H, $\text{ArH}_{f=f', g}$), 7.75–7.79 (m, 2H, $\text{ArH}_{e=e'}$), ^{13}C NMR (75 MHz, CDCl_3) δ 14.8, 22.0, 42.3, 59.4, 63.4, 114.7 (2C), 126.5 (2C), 126.9 (2C), 128.7 (2C), 130.3, 131.4, 133.9, 153.8, 158.3, 168.8; (EI) m/z (M^{+} 308, Base Peak 265). Anal. calcd. for $\text{C}_{19}\text{H}_{20}\text{N}_2\text{O}_2$: C, 74.00; H, 6.54; N, 9.08; Found: C, 73.94; H, 6.49; N, 9.17 %.

1-Acetyl-3-phenyl-5-(4-propyloxyphenyl)-2-pyrazoline (1c)

Yellowish white crystals. Yield 83%. M. p. 114–116 °C; $R_f = 0.72$ (petroleum ether:ethyl acetate, 4:1), FT-IR (KBr, cm^{-1}) 1679, 1644, 1489, 1297, 1252, 1043, ^1H NMR (300 MHz, CDCl_3) δ 1.03 (t, 3H, $J = 7.5$ Hz, $-\text{O}-(\text{CH}_2)_2-\text{CH}_3$), 1.80 (sextet 2H, $J = 7.5$ Hz, $-\text{O}-\text{CH}_2-\text{CH}_2-\text{CH}_3$), 2.43 (s, 3H, $\text{O}=\text{C}-\text{CH}_3$), 3.18 (dd, 1H, $J = 4.5, 17.7$ Hz, H_a), 3.75 (dd, 1H, $J = 11.7, 17.7$ Hz, H_b), 3.90 (t, 2H, $J = 6.6$ Hz, $-\text{O}-\text{CH}_2-$), 5.57 (dd, 1H, $J = 4.5, 11.7$ Hz, H_x), 6.85 (d, 2H, $J = 8.7$ Hz, $\text{ArH}_{c=c'}$), 7.17 (d, 2H, $J = 8.7$ Hz, $\text{ArH}_{d=d'}$), 7.44–7.47 (m, 3H, $\text{ArH}_{f=f', g}$), 7.75–7.79 (m, 2H, $\text{ArH}_{e=e'}$), ^{13}C NMR (75 MHz, CDCl_3) δ 10.5, 22.0, 22.5, 42.2, 59.4, 69.5, 114.7 (2C), 126.5 (2C), 126.9 (2C), 128.7 (2C), 130.2, 131.5, 133.9, 153.8, 158.6, 168.8, (EI) m/z (M^{+} 322, Base Peak 279). Anal. calcd. for $\text{C}_{20}\text{H}_{22}\text{N}_2\text{O}_2$: C, 74.51; H, 6.88; N, 8.69; Found: C, 74.48; H, 6.81; N, 8.78 %.

1-Acetyl-3-phenyl-5-(4-butyloxyphenyl)-2-pyrazoline (1d)

Yellowish white crystals. Yield 86%. M. p. 88–90 °C; $R_f = 0.71$ (petroleum ether:ethyl acetate, 4:1), FT-IR (KBr, cm^{-1}) 1675, 1642, 1490, 1292, 1259, 1048, ^1H NMR (300 MHz, CDCl_3) δ 0.95 (t, 3H, $J = 7.5$ Hz, $-\text{O}-(\text{CH}_2)_3-\text{CH}_3$), 1.48 (sextet 2H, $J = 7.8$ Hz, $-\text{O}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_3$), 1.76 (qn 2H, $J = 7.0$ Hz, $-\text{O}-\text{CH}_2-\text{CH}_2-\text{C}_2\text{H}_5$), 2.43 (s, 3H, $\text{O}=\text{C}-\text{CH}_3$), 3.18 (dd, 1H, $J = 4.5, 17.7$ Hz, H_a), 3.74 (dd, 1H, $J = 11.7, 17.7$ Hz, H_b), 3.94 (t, 2H, $J = 6.6$ Hz, $-\text{O}-\text{CH}_2-$), 5.57 (dd, 1H, $J = 4.5, 12.0$ Hz, H_x), 6.85 (d, 2H, $J = 8.7$ Hz, $\text{ArH}_{c=c'}$), 7.17 (d, 2H, $J = 8.7$ Hz, $\text{ArH}_{d=d'}$), 7.44–7.47 (m, 3H, $\text{ArH}_{f=f', g}$), 7.75–7.79 (m, 2H, $\text{ArH}_{e=e'}$), ^{13}C NMR (75 MHz, CDCl_3) δ 13.8, 19.2, 22.0, 31.2, 42.2, 59.4, 67.6, 114.7 (2C), 126.5 (2C), 126.9 (2C), 128.7 (2C), 130.2, 131.5, 133.8, 153.8, 158.6, 168.8, (EI) m/z (M^{+} 336, Base Peak 293). Anal. calcd. for $\text{C}_{21}\text{H}_{24}\text{N}_2\text{O}_2$: C, 74.97; H, 7.19; N, 8.33; Found: C, 74.92; H, 7.15; N, 8.41 %.

1-Acetyl-3-phenyl-5-(4-pentyloxyphenyl)-2-pyrazoline (1e)

Yellowish white crystals. Yield 87%. M. p. 85–87 °C; $R_f =$

0.68 (petroleum ether:ethyl acetate, 4:1), FT-IR (KBr, cm^{-1}) 1682, 1636, 1493, 1293, 1253, 1045, ^1H NMR (300 MHz, CDCl_3) δ 0.94 (t, 3H, $J = 7.0$ Hz, $-\text{O}-(\text{CH}_2)_4-\text{CH}_3$), 1.35–1.46 (m 4H, $J = 7.8$ Hz, $-\text{O}-\text{CH}_2-\text{CH}_2-(\text{CH}_2)_2-\text{CH}_3$), 1.77 (qn 2H, $J = 7.0$ Hz, $-\text{O}-\text{CH}_2-\text{CH}_2-\text{C}_3\text{H}_7$), 2.43 (s, 3H, $\text{O}=\text{C}-\text{CH}_3$), 3.18 (dd, 1H, $J = 4.5, 17.7$ Hz, H_a), 3.74 (dd, 1H, $J = 11.7, 17.4$ Hz, H_b), 3.92 (t, 2H, $J = 6.6$ Hz, $-\text{O}-\text{CH}_2-$), 5.57 (dd, 1H, $J = 4.5, 11.7$ Hz, H_x), 6.85 (d, 2H, $J = 8.7$ Hz, $\text{ArH}_{c=c'}$), 7.17 (d, 2H, $J = 8.7$ Hz, $\text{ArH}_{d=d'}$), 7.44–7.47 (m, 3H, $\text{ArH}_{f=f', g}$), 7.75–7.79 (m, 2H, $\text{ArH}_{e=e'}$), ^{13}C NMR (75 MHz, CDCl_3) δ 14.6, 22.0, 22.4, 28.1, 28.9, 42.3, 59.4, 67.9, 114.7 (2C), 126.5 (2C), 126.9 (2C), 128.7 (2C), 130.2, 131.5, 133.8, 153.8, 158.6, 168.8, (EI) m/z (M^{+} 350, Base Peak 307). Anal. calcd. for $\text{C}_{22}\text{H}_{26}\text{N}_2\text{O}_2$: C, 75.40; H, 7.48; N, 7.99; Found: C, 75.36; H, 7.42; N, 8.07 %.

1-Acetyl-3-phenyl-5-(4-hexyloxyphenyl)-2-pyrazoline (1f)

Yellowish white crystals. Yield 83%. M.p. 82–85 °C; $R_f = 0.69$ (petroleum ether:ethyl acetate, 4:1), FT-IR (KBr, cm^{-1}) 1676, 1634, 1497, 1295, 1250, 1049, ^1H NMR (300 MHz, CDCl_3) δ 0.91 (t, 3H, $J = 7.0$ Hz, $-\text{O}-(\text{CH}_2)_5-\text{CH}_3$), 1.31–1.47 (m 6H, $-\text{O}-\text{CH}_2-\text{CH}_2-(\text{CH}_2)_3-\text{CH}_3$), 1.76 (qn 2H, $J = 7.0$ Hz, $-\text{O}-\text{CH}_2-\text{CH}_2-\text{C}_4\text{H}_9$), 2.43 (s, 3H, $\text{O}=\text{C}-\text{CH}_3$), 3.18 (dd, 1H, $J = 4.8, 17.7$ Hz, H_a), 3.74 (dd, 1H, $J = 11.7, 17.7$ Hz, H_b), 3.93 (t, 2H, $J = 6.6$ Hz, $-\text{O}-\text{CH}_2-$), 5.57 (dd, 1H, $J = 4.2, 11.7$ Hz, H_x), 6.85 (d, 2H, $J = 8.7$ Hz, $\text{ArH}_{c=c'}$), 7.17 (d, 2H, $J = 8.7$ Hz, $\text{ArH}_{d=d'}$), 7.44–7.47 (m, 3H, $\text{ArH}_{f=f', g}$), 7.75–7.79 (m, 2H, $\text{ArH}_{e=e'}$), ^{13}C NMR (75 MHz, CDCl_3) δ 14.0, 22.0, 22.6, 25.7, 29.2, 31.5, 42.3, 59.4, 67.9, 114.7 (2C), 126.5 (2C), 126.9 (2C), 128.7 (2C), 130.2, 131.5, 133.8, 153.8, 158.6, 168.8, (EI) m/z (M^{+} 364, Base Peak 321). Anal. calcd. for $\text{C}_{23}\text{H}_{28}\text{N}_2\text{O}_2$: C, 75.79; H, 7.74; N, 7.69; Found: C, 75.74; H, 7.69; N, 7.78 %.

1-Acetyl-3-phenyl-5-(4-heptyloxyphenyl)-2-pyrazoline (1g)

Yellowish white crystals. Yield 89%. M.p. 89–91 °C; $R_f = 0.71$ (petroleum ether:ethyl acetate, 4:1), FT-IR (KBr, cm^{-1}) 1678, 1639, 1487, 1291, 1256, 1052, ^1H NMR (300 MHz, CDCl_3) δ 0.91 (t, 3H, $J = 7.0$ Hz, $-\text{O}-(\text{CH}_2)_6-\text{CH}_3$), 1.32–1.47 (m 8H, $-\text{O}-\text{CH}_2-\text{CH}_2-(\text{CH}_2)_4-\text{CH}_3$), 1.77 (qn 2H, $J = 7.0$ Hz, $-\text{O}-\text{CH}_2-\text{CH}_2-\text{C}_5\text{H}_{11}$), 2.43 (s, 3H, $\text{O}=\text{C}-\text{CH}_3$), 3.18 (dd, 1H, $J = 4.5, 17.7$ Hz, H_a), 3.74 (dd, 1H, $J = 11.7, 17.7$ Hz, H_b), 3.93 (t, 2H, $J = 6.6$ Hz, $-\text{O}-\text{CH}_2-$), 5.57 (dd, 1H, $J = 4.5, 12.0$ Hz, H_x), 6.85 (d, 2H, $J = 8.7$ Hz, $\text{ArH}_{c=c'}$), 7.17 (d, 2H, $J = 8.7$ Hz, $\text{ArH}_{d=d'}$), 7.44–7.47 (m, 3H, $\text{ArH}_{f=f', g}$), 7.75–7.79 (m, 2H, $\text{ArH}_{e=e'}$), ^{13}C NMR (75 MHz, CDCl_3) δ 14.1, 22.0, 22.6, 26.0, 29.0, 29.2, 31.7, 42.2, 59.4, 68.0, 114.7 (2C), 126.5 (2C), 126.9 (2C), 128.7 (2C), 130.2, 131.5, 133.8, 153.8, 158.6, 168.8, (EI) m/z (M^{+} 378, Base Peak 335). Anal. calcd. for $\text{C}_{24}\text{H}_{30}\text{N}_2\text{O}_2$: C, 76.16; H, 7.99; N, 7.40; Found: C, 76.12; H, 7.91; N, 7.49 %.

1-Acetyl-3-phenyl-5-(4-octyloxyphenyl)-2-pyrazoline (1h)

Yellowish white crystals. Yield 82%. M.p. 73–75 °C; R_f = 0.72 (petroleum ether:ethyl acetate, 4:1), FT-IR (KBr, cm^{-1}) 1681, 1645, 1492, 1297, 1258, 1056, ^1H NMR (300 MHz, CDCl_3) δ 0.90 (t, 3H, J = 7.0 Hz, $-\text{O}-(\text{CH}_2)_7-\text{CH}_3$), 1.29–1.49 (m 10H, $-\text{O}-\text{CH}_2-\text{CH}_2-(\text{CH}_2)_5-\text{CH}_3$), 1.76 (qn 2H, J = 7.8 Hz, $-\text{O}-\text{CH}_2-\text{CH}_2-\text{C}_6\text{H}_{13}$), 2.43 (s, 3H, $\text{O}=\text{C}-\text{CH}_3$), 3.18 (dd, 1H, J = 4.5, 17.7 Hz, H_a), 3.74 (dd, 1H, J = 11.7, 17.4 Hz, H_b), 3.92 (t, 2H, J = 6.6 Hz, $-\text{O}-\text{CH}_2-$), 5.57 (dd, 1H, J = 4.5, 11.7 Hz, H_x), 6.85 (d, 2H, J = 8.7 Hz, $\text{ArH}_{c=c'}$), 7.17 (d, 2H, J = 8.7 Hz, $\text{ArH}_{d=d'}$), 7.44–7.47 (m, 3H, $\text{ArH}_{f=f',g}$), 7.75–7.79 (m, 2H, $\text{ArH}_{e=e'}$), ^{13}C NMR (75 MHz, CDCl_3) δ 14.3, 22.4, 22.6, 26.0, 29.1, 29.2, 29.3, 31.8, 42.3, 59.4, 67.9, 114.7 (2C), 126.5 (2C), 126.9 (2C), 128.7 (2C), 130.2, 131.5, 133.8, 153.8, 158.6, 168.8, (EI) m/z (M^{+} 392, Base Peak 349). Anal. calcd. for $\text{C}_{25}\text{H}_{32}\text{N}_2\text{O}_2$: C, 76.49; H, 8.22; N, 7.14; Found: C, 76.44; H, 8.18; N, 7.21%.

1- Propionyl-3-phenyl-5-(4-methoxyphenyl)-2-pyrazoline (2a)

Yellowish white crystals. Yield 81%. M.p. 100–103 °C; R_f = 0.73 (petroleum ether:ethyl acetate, 4:1), FT-IR (KBr, cm^{-1}) 1685, 1635, 1493, 1294, 1254, 1049, ^1H NMR (300 MHz, CDCl_3) δ 1.22 (t, 3H, J = 7.5 Hz, $\text{O}=\text{C}-\text{CH}_2-\text{CH}_3$), 2.84 (q, 2H, J = 7.5 Hz, $\text{O}=\text{C}-\text{CH}_2-\text{CH}_3$), 3.17 (dd, 1H, J = 4.8, 17.7 Hz, H_a), 3.73 (dd, 1H, J = 12.0, 17.7 Hz, H_b), 3.79 (s, 3H, $-\text{O}-\text{CH}_3$), 5.56 (dd, 1H, J = 4.8, 11.7 Hz, H_x), 6.86 (d, 2H, J = 8.7 Hz, $\text{ArH}_{c=c'}$), 7.19 (d, 2H, J = 8.7 Hz, $\text{ArH}_{d=d'}$), 7.44–7.46 (m, 3H, $\text{ArH}_{f=f',g}$), 7.76–7.79 (m, 2H, $\text{ArH}_{e=e'}$), ^{13}C NMR (75 MHz, CDCl_3) δ 9.0, 27.6, 42.0, 55.2, 59.6, 114.2 (2C), 126.5 (2C), 126.9 (2C), 128.7 (2C), 130.2, 131.6, 134.3, 153.5, 158.9, 172.2, (EI) m/z (M^{+} 308, Base Peak 252). Anal. calcd. for $\text{C}_{19}\text{H}_{20}\text{N}_2\text{O}_2$: C, 74.00; H, 6.54; N, 9.08; Found: C, 73.93; H, 6.48; N, 9.19%.

1- Propionyl-3-phenyl-5-(4-ethoxyphenyl)-2-pyrazoline (2b)

Yellowish white crystals. Yield 85%. M.p. 98–101 °C; R_f = 0.70 (petroleum ether:ethyl acetate, 4:1), FT-IR (KBr, cm^{-1}) 1688, 1637, 1497, 1298, 1252, 1045, ^1H NMR (300 MHz, CDCl_3) δ 1.20 (t, 3H, J = 7.5 Hz, $\text{O}=\text{C}-\text{CH}_2-\text{CH}_3$), 1.40 (t, 3H, J = 7.2 Hz, $-\text{O}-\text{CH}_2-\text{CH}_3$), 2.83 (q, 2H, J = 7.5 Hz, $\text{O}=\text{C}-\text{CH}_2-\text{CH}_3$), 3.16 (dd, 1H, J = 4.8, 17.7 Hz, H_a), 3.72 (dd, 1H, J = 11.7, 17.4 Hz, H_b), 4.00 (q, 2H, J = 7.4 Hz, $-\text{O}-\text{CH}_2-\text{CH}_3$), 5.55 (dd, 1H, J = 4.5, 11.7 Hz, H_x), 6.84 (d, 2H, J = 8.7 Hz, $\text{ArH}_{c=c'}$), 7.17 (d, 2H, J = 8.7 Hz, $\text{ArH}_{d=d'}$), 7.43–7.45 (m, 3H, $\text{ArH}_{f=f',g}$), 7.75–7.79 (m, 2H, $\text{ArH}_{e=e'}$), ^{13}C NMR (75 MHz, CDCl_3) δ 9.0, 14.8, 27.6, 42.0, 59.6, 63.4, 114.7 (2C), 126.5 (2C), 126.9 (2C), 128.7 (2C), 130.2, 131.6, 134.1, 153.6, 158.3, 172.3, (EI) m/z (M^{+} 322, Base Peak 265). Anal. calcd. for $\text{C}_{20}\text{H}_{22}\text{N}_2\text{O}_2$: C, 74.51; H, 6.88; N, 8.69; Found: C, 74.46; H, 6.78; N, 8.79%.

1- Propionyl-3-phenyl-5-(4-propyloxyphenyl)-2-pyrazoline (2c)

Yellowish white crystals. Yield 88%. M.p. 107–109 °C; R_f = 0.71 (petroleum ether:ethyl acetate, 4:1), FT-IR (KBr, cm^{-1}) 1683, 1641, 1490, 1296, 1255, 1047, ^1H NMR (300 MHz, CDCl_3) δ 1.03 (t, 3H, J = 7.5 Hz, $-\text{O}-(\text{CH}_2)_2-\text{CH}_3$), 1.21 (t, 3H, J = 7.8 Hz, $\text{O}=\text{C}-\text{CH}_2-\text{CH}_3$), 1.80 (sextet, 2H, J = 7.2 Hz, $-\text{O}-\text{CH}_2-\text{CH}_2-\text{CH}_3$), 2.83 (q, 2H, J = 7.5 Hz, $\text{O}=\text{C}-\text{CH}_2-\text{CH}_3$), 3.17 (dd, 1H, J = 4.5, 17.7 Hz, H_a), 3.73 (dd, 1H, J = 11.7, 17.4 Hz, H_b), 3.90 (t, 2H, J = 6.6 Hz, $-\text{O}-\text{CH}_2-\text{CH}_2-\text{CH}_3$), 5.55 (dd, 1H, J = 4.8, 11.7 Hz, H_x), 6.85 (d, 2H, J = 8.7 Hz, $\text{ArH}_{c=c'}$), 7.17 (d, 2H, J = 8.7 Hz, $\text{ArH}_{d=d'}$), 7.44–7.46 (m, 3H, $\text{ArH}_{f=f',g}$), 7.76–7.79 (m, 2H, $\text{ArH}_{e=e'}$), ^{13}C NMR (75 MHz, CDCl_3) δ 9.0, 10.5, 22.5, 27.6, 42.0, 59.6, 69.4, 114.7 (2C), 126.5 (2C), 126.9 (2C), 128.7 (2C), 130.2, 131.6, 134.1, 153.5, 158.5, 172.2, (EI) m/z (M^{+} 336, Base Peak 280). Anal. calcd. for $\text{C}_{21}\text{H}_{24}\text{N}_2\text{O}_2$: C, 74.97; H, 7.19; N, 8.33; Found: C, 74.91; H, 7.16; N, 8.42%.

1- Propionyl-3-phenyl-5-(4-butyloxyphenyl)-2-pyrazoline (2d)

Yellowish white crystals. Yield 86%. M.p. 104–107 °C; R_f = 0.69 (petroleum ether:ethyl acetate, 4:1), FT-IR (KBr, cm^{-1}) 1677, 1645, 1494, 1290, 1257, 1051, ^1H NMR (300 MHz, CDCl_3) δ 0.95 (t, 3H, J = 7.0 Hz, $-\text{O}-(\text{CH}_2)_3-\text{CH}_3$), 1.21 (t, 3H, J = 7.5 Hz, $\text{O}=\text{C}-\text{CH}_2-\text{CH}_3$), 1.47 (sextet 2H, J = 7.8 Hz, $-\text{O}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_3$), 1.76 (qn 2H, J = 7.0 Hz, $-\text{O}-\text{CH}_2-\text{CH}_2-\text{C}_2\text{H}_5$), 2.83 (q, 2H, J = 7.5 Hz, $\text{O}=\text{C}-\text{CH}_2-\text{CH}_3$), 3.18 (dd, 1H, J = 4.5, 17.7 Hz, H_a), 3.74 (dd, 1H, J = 11.7, 17.7 Hz, H_b), 3.94 (t, 2H, J = 6.6 Hz, $-\text{O}-\text{CH}_2-$), 5.57 (dd, 1H, J = 4.5, 12.0 Hz, H_x), 6.85 (d, 2H, J = 8.7 Hz, $\text{ArH}_{c=c'}$), 7.17 (d, 2H, J = 8.7 Hz, $\text{ArH}_{d=d'}$), 7.44–7.47 (m, 3H, $\text{ArH}_{f=f',g}$), 7.75–7.79 (m, 2H, $\text{ArH}_{e=e'}$), ^{13}C NMR (75 MHz, CDCl_3) δ 9.0, 12.5, 22.5, 27.6, 28.1, 42.0, 59.6, 68.4, 114.7 (2C), 126.5 (2C), 126.9 (2C), 128.7 (2C), 130.2, 131.5, 134.1, 153.5, 158.5, 172.4, (EI) m/z (M^{+} 350, Base Peak 294). Anal. calcd. for $\text{C}_{22}\text{H}_{26}\text{N}_2\text{O}_2$: C, 75.40; H, 7.48; N, 7.99; Found: C, 75.33; H, 7.45; N, 8.08%.

1- Propionyl-3-phenyl-5-(4-pentyloxyphenyl)-2-pyrazoline (2e)

Yellowish white crystals. Yield 85%. M.p. 101–103 °C; R_f = 0.72 (petroleum ether:ethyl acetate, 4:1), FT-IR (KBr, cm^{-1}) 1681, 1639, 1492, 1297, 1255, 1053, ^1H NMR (300 MHz, CDCl_3) δ 0.94 (t, 3H, J = 7.0 Hz, $-\text{O}-(\text{CH}_2)_4-\text{CH}_3$), 1.21 (t, 3H, J = 7.5 Hz, $\text{O}=\text{C}-\text{CH}_2-\text{CH}_3$), 1.35–1.48 (m 4H, $-\text{O}-\text{CH}_2-\text{CH}_2-(\text{CH}_2)_2-\text{CH}_3$), 1.77 (qn 2H, J = 7.0 Hz, $-\text{O}-\text{CH}_2-\text{CH}_2-\text{C}_3\text{H}_7$), 2.83 (q, 2H, J = 7.5 Hz, $\text{O}=\text{C}-\text{CH}_2-\text{CH}_3$), 3.16 (dd, 1H, J = 4.8, 17.7 Hz, H_a), 3.72 (dd, 1H, J = 11.7, 17.7 Hz, H_b), 3.93 (t, 2H, J = 6.6 Hz, $-\text{O}-\text{CH}_2-$), 5.55 (dd, 1H, J = 4.5, 11.7 Hz, H_x), 6.84 (d, 2H, J = 8.7 Hz, $\text{ArH}_{c=c'}$), 7.17 (d, 2H, J = 8.7 Hz, $\text{ArH}_{d=d'}$), 7.43–7.47 (m, 3H, $\text{ArH}_{f=f',g}$), 7.75–7.78 (m, 2H, $\text{ArH}_{e=e'}$), ^{13}C NMR (75 MHz, CDCl_3) δ 9.0, 14.0, 22.4, 27.6, 28.1,

28.9, 42.0, 59.6, 67.9, 114.7 (2C), 126.5 (2C), 126.9 (2C), 128.7 (2C), 130.1, 131.6, 134.0, 153.5, 158.5, 172.2, (EI) m/z (M^{+} 364, Base Peak 308). Anal. calcd. for $C_{23}H_{28}N_2O_2$: C, 75.79; H, 7.74; N, 7.69; Found: C, 75.73; H, 7.71; N, 7.78%.

1-Propionyl-3-phenyl-5-(4-hexyloxyphenyl)-2-pyrazoline (2f)

Yellowish white crystals. Yield 82%. M.p. 95–98 °C; R_f = 0.68 (petroleum ether:ethyl acetate, 4:1), FT-IR (KBr, cm^{-1}) 1679, 1638, 1498, 1293, 1253, 1045, 1H NMR (300 MHz, $CDCl_3$) δ 0.92 (t, 3H, J = 7.0 Hz, $-O-(CH_2)_5-CH_3$), 1.21 (t, 3H, J = 7.5 Hz, $O=C-CH_2-CH_3$), 1.31–1.47 (m 6H, $-O-CH_2-CH_2-(CH_2)_3-CH_3$), 1.77 (qn 2H, J = 7.5 Hz, $-O-CH_2-CH_2-C_6H_9$), 2.83 (q, 2H, J = 7.5 Hz, $O=C-CH_2-CH_3$), 3.17 (dd, 1H, J = 4.8, 17.7 Hz, H_a), 3.73 (dd, 1H, J = 11.7, 17.7 Hz, H_b), 3.93 (t, 2H, J = 6.6 Hz, $-O-CH_2-$), 5.55 (dd, 1H, J = 4.8, 12.0 Hz, H_x), 6.84 (d, 2H, J = 8.7 Hz, $ArH_{c=c'}$), 7.17 (d, 2H, J = 8.7 Hz, $ArH_{d=d'}$), 7.43–7.47 (m, 3H, $ArH_{f=f', g}$), 7.74–7.79 (m, 2H, $ArH_{e=e'}$), ^{13}C NMR (75 MHz, $CDCl_3$) δ 9.0, 14.0, 22.6, 25.7, 27.6, 29.2, 31.5, 42.0, 59.6, 67.9, 114.7 (2C), 126.5 (2C), 126.9 (2C), 128.7 (2C), 130.1, 131.6, 134.0, 153.5, 158.5, 172.2, (EI) m/z (M^{+} 378, Base Peak 322). Anal. calcd. for $C_{24}H_{30}N_2O_2$: C, 76.16; H, 7.99; N, 7.40; Found: C, 76.09; H, 7.91; N, 7.51%.

1-Propionyl-3-phenyl-5-(4-heptyloxyphenyl)-2-pyrazoline (2g)

Yellowish white crystals. Yield 84%. M.p. 90–92 °C; R_f = 0.71 (petroleum ether:ethyl acetate, 4:1), FT-IR (KBr, cm^{-1}) 1678, 1633, 1489, 1296, 1252, 1048, 1H NMR (300 MHz, $CDCl_3$) δ 0.91 (t, 3H, J = 7.0 Hz, $-O-(CH_2)_6-CH_3$), 1.21 (t, 3H, J = 7.5 Hz, $O=C-CH_2-CH_3$), 1.32–1.47 (m 8H, $-O-CH_2-CH_2-(CH_2)_4-CH_3$), 1.77 (qn 2H, J = 7.8 Hz, $-O-CH_2-CH_2-C_5H_{11}$), 2.83 (q, 2H, J = 7.5 Hz, $O=C-CH_2-CH_3$), 3.17 (dd, 1H, J = 4.8, 17.7 Hz, H_a), 3.73 (dd, 1H, J = 11.7, 17.4 Hz, H_b), 3.93 (t, 2H, J = 6.6 Hz, $-O-CH_2-$), 5.55 (dd, 1H, J = 4.5, 11.7 Hz, H_x), 6.84 (d, 2H, J = 8.7 Hz, $ArH_{c=c'}$), 7.17 (d, 2H, J = 8.7 Hz, $ArH_{d=d'}$), 7.44–7.46 (m, 3H, $ArH_{f=f', g}$), 7.76–7.79 (m, 2H, $ArH_{e=e'}$), ^{13}C NMR (75 MHz, $CDCl_3$) δ 9.0, 14.1, 22.6, 26.0, 27.6, 29.0, 29.2, 31.8, 42.0, 59.6, 67.9, 114.7 (2C), 126.5 (2C), 126.9 (2C), 128.7 (2C), 130.2, 131.6, 134.0, 153.5, 158.5, 172.2, (EI) m/z (M^{+} 392, Base Peak 336). Anal. calcd. for $C_{25}H_{32}N_2O_2$: C, 76.49; H, 8.22; N, 7.14; Found: C, 76.43; H, 8.18; N, 7.23%.

1-Propionyl-3-phenyl-5-(4-octyloxyphenyl)-2-pyrazoline (2h)

Yellowish white crystals. Yield 87%. M.p. 73–75 °C; R_f = 0.73 (petroleum ether:ethyl acetate, 4:1), FT-IR (KBr, cm^{-1}) 1682, 1647, 1495, 1294, 1255, 1051, 1H NMR (300 MHz, $CDCl_3$) δ 0.91 (t, 3H, J = 7.0 Hz, $-O-(CH_2)_7-CH_3$), 1.21 (t, 3H, J = 7.5 Hz, $O=C-CH_2-CH_3$), 1.31–1.46 (m 10H, $-O-CH_2-CH_2-(CH_2)_5-CH_3$), 1.77 (qn 2H, J = 7.8

Hz, $-O-CH_2-CH_2-C_6H_{13}$), 2.83 (q, 2H, J = 7.5 Hz, $O=C-CH_2-CH_3$), 3.17 (dd, 1H, J = 4.8, 17.7 Hz, H_a), 3.72 (dd, 1H, J = 11.7, 17.4 Hz, H_b), 3.93 (t, 2H, J = 6.6 Hz, $-O-CH_2-$), 5.55 (dd, 1H, J = 4.5, 11.7 Hz, H_x), 6.84 (d, 2H, J = 8.7 Hz, $ArH_{c=c'}$), 7.17 (d, 2H, J = 8.7 Hz, $ArH_{d=d'}$), 7.44–7.46 (m, 3H, $ArH_{f=f', g}$), 7.76–7.79 (m, 2H, $ArH_{e=e'}$), ^{13}C NMR (75 MHz, $CDCl_3$) δ 9.0, 9.4, 14.1, 22.6, 26.0, 27.6, 29.2, 29.3, 31.8, 42.0, 59.6, 67.9, 114.7 (2C), 126.5 (2C), 126.9 (2C), 128.7 (2C), 130.2, 131.6, 134.0, 153.5, 158.5, 172.2, (EI) m/z (M^{+} 406, Base Peak 350). Anal. calcd. for $C_{26}H_{34}N_2O_2$: C, 76.81; H, 8.43; N, 6.89; Found: C, 76.77; H, 8.39; N, 6.96%.

4. 3. Anti-inflammatory Activity (*in vitro*)

Inflammation occurs as a defensive response, which induces physiological adaptations to limit tissue damage and removes the pathogenic infection. Reactive oxygen species (ROS) are formed subsequent to the assembly and activation of the phagocyte-specific enzyme, NADPH Oxidase. This process is initiated by the production of superoxide anion during a ‘respiratory burst’ of non-mitochondrial oxygen uptake by an NADPH oxidase system. This study used the water-soluble tetrazolium salt (WST-1) to measure superoxide production by neutrophils activated by opsonized zymosan, which induces phagocytic activation of neutrophils.⁴⁷ This technique is more sensitive and reliable as compared to other available techniques.

4. 3. 1. Respiratory Burst Assay

Anti-inflammatory activity of the test compounds was determined by using a modified assay of Tan & Beridge.⁴⁵ This *in vitro* assay was based on the reduction of highly water-soluble tetrazolium salt (WST-1) in the presence of activated neutrophils. Anti-inflammatory activity was determined in a total volume of 200 μ L MHS (pH 7.4) containing 1.0–104 neutrophils/mL, 250 μ M WST-1 and various concentrations of test compounds. The control contained buffer, neutrophils and WST-1. All compounds were equilibrated at 37 °C and the reaction was initiated by adding opsonized zymosan A (15 mg/mL), which was prepared by mixing with human pooled serum, followed by centrifugation at 3000 rpm whereby the pellet was resuspended in PBS buffer. Absorbance was measured at 450 nm. Aspirin and indomethacin were used as positive controls which are widely used as non-steroidal anti-inflammatory drugs (NSAIDs) for the treatment of several inflammatory diseases. Values of IC_{50} were calculated by comparison with the DMSO as the blank and expressed as the percent inhibition of superoxide anions produced. The percent inhibitory activity by the samples was determined against a DMSO blank and calculated using the following formula: % Inhibition = $100 - [(OD \text{ test compound} / OD \text{ control}) \times 100]$ IC_{50} of samples was determined by using EZ-FIT Windows-based software.

4.4. Cytotoxicity Assay Against PC-3 Cell Line (Prostate Cancer)

Cytotoxic activity of the synthesized compounds was evaluated in 96-well flat-bottomed micro plates by using the standard MTT (3-[4, 5-dimethylthiazole-2-yl]-2,5-diphenyl-tetrazolium bromide) colorimetric assay.⁴⁶ For this purpose, PC-3 cells (Prostate Cancer) were cultured in Dulbecco's Modified Eagle's Medium, supplemented with 5% of fetal bovine serum (FBS), 100 IU/mL of penicillin and 100 µg/mL of streptomycin in 25 cm³ flask, and kept in 5% CO₂ incubator at 37 °C. Exponentially growing cells were harvested, counted with haemocytometer and diluted with a particular medium. Cell culture with the concentration of 1 × 10⁵ cells/mL was prepared and introduced (100 µL/well) into 96-well plates. After overnight incubation, medium was removed and 200 µL of fresh medium was added with different concentrations of compounds (1–100 µM). After 48 h, 50 µL MTT (2 mg/mL) was added to each well and incubated further for 4 hrs. Subsequently, 100 µL of DMSO was added to each well. The extent of MTT reduction to formazan within cells was calculated by measuring the absorbance at 570 nm, using a micro plate reader (Spectra Max plus, Molecular Devices, CA, USA). The cytotoxicity was recorded as concentration causing 50% growth inhibition (IC₅₀) for PC3.

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Povzetek

Sintetizirali smo serijo novih pirazolinskih derivativov **1a–2h**, ki vsebujejo *N*-acilne skupine in homologne alkiloksi stranske verige. Karakterizacija novih spojin je temeljila na spektroskopskih in mikroanalitskih rezultatih. Vse pripravljene spojine smo *in vitro* testirali za njihovo proti vnetno učinkovanje; zanimal nas je predvsem vpliv dolžine alkiloksi stranske verige na aktivnost. Izkazalo se je, da spojine z lihim številom ogljikovih atomov v alkiloksi stranski verigi izkazujejo boljšo aktivnost kot spojine s sodim številom ogljikov. Spojina **2c** (96% inhibicija, $IC_{50} = 173.06 \pm 2.312$ mM) se je izkazala kot najbolj aktivna izmed vseh spojin v seriji; bila je celo bolj aktivna kot standardna učinkovina (indometacin, 92% inhibicija, $IC_{50} = 273.12 \pm 2.33$ mM). Spojina **1a** (86%, $IC_{50} = 296.16 \pm 2.091$ mM) je bila druga najbolj učinkovita z aktivnostjo, primerljivo z aktivnostjo standardne učinkovine. Ostale spojine v seriji pa so pokazale le zmereno do nizko aktivnost. Zanimivo pa je, da so vzporedne študije citotoksičnosti spojin **1a–2h** proti celični liniji PC-3 pokazale zelo majhno ali celo ničelno aktivnost. Pričujoča študija bo morda lahko prispevala k razvoju novih alternativnih učinkovin trenutno uporabljanim nesteroidnim proti vnetnim učinkovinam (NSAID), ki zaradi neposredne citotoksičnosti kažejo škodljive vplive na prebavila.